

REGULATION OF GROWTH IN RED KITES AND IMPERIAL EAGLES

JAVIER VIÑUELA¹ AND MIGUEL FERRER²

ABSTRACT.—We analyzed variability in growth of Red Kite (*Milvus milvus*) chicks using biometrical measures and urea and alkaline phosphatase blood levels. We used variables estimating relative mass and tarsus length growth rates during the 5–7 days before every blood extraction (short-term growth) and deviations of size (mass and tarsus length) from population averages at the age of blood extraction (long-term growth). We found a significant relationship between blood levels of alkaline phosphatase and short-term tarsus growth rate but not with long-term tarsus growth. Urea levels were correlated with long-term mass growth but not with short-term growth. We found some chicks with relatively rapid mass growth, slow tarsus growth, and low levels of alkaline phosphatase after periods of fasting. Chicks of Imperial Eagles (*Aquila adalberti*) showed less variable nestling periods but higher and more variable blood urea levels. These differences agree with theoretical predictions about the evolution of growth regulation abilities in relation to feeding ecology: the species with a more variable growth and more resistance to food deprivation can experience a more irregular and unpredictable food supply. Received 4 Dec. 1995, accepted 10 April 1996.

Two hypotheses have been proposed to explain patterns of growth in birds. Ricklefs (1968) proposed that chicks grow at the maximal rate allowed by food intake. Physiological or cellular constraints would determine theoretical maximum growth rate (Maximization Hypothesis). Case (1978) criticized this hypothesis, arguing that rapid growth could be associated with higher mortality rates and proposed that growth rates could be adapted to the ecological features of species (optimization hypothesis; also see O'Connor 1978). These are not mutually exclusive hypotheses, but their relative importance could vary between species depending on ecological constraints (Ricklefs 1984). The optimization hypothesis assumes that growth rates may be maintained below a given level by active control (Callow and Townsend 1981) and predicts that the degree of variability and control of growth rates depends mainly on the predictability and stability of food resources during the nestling period (O'Connor 1978). It is well known that growth rates can be affected by a large variety of environmental factors (Ricklefs 1983). In species with a small clutch size and unpredictable food resources at the time of laying, variable growth rates may work as a fine-tuning mechanism to regulate productivity (O'Connor 1984, Donazar and Ceballos 1989, Veiga and Hiraldo 1990).

Although growth in birds has been extensively studied by biometrical

¹ Museo Nacional de Ciencias Naturales, CSIC, José Gutierrez Abascal 2, 28006-Madrid, Spain.

² Estación Biológica de Doñana, CSIC, Avda. Maria Luisa s/n, 41013-Sevilla, Spain.

and observational procedures, experimental and physiological studies are relatively less common (Ricklefs 1983), and hematological indices have rarely been applied to the study of patterns of growth of wild birds (but see Cherel et al. 1987).

In this paper we analyze growth variability in the Red Kite (*Milvus milvus*), using biometrical and physiological methodologies, and we explore the possible existence of mechanisms to regulate growth rate. We compare variables between Red Kites and Spanish Imperial Eagles (*Aquila adalberti*). The Red Kite is an opportunistic predator (Delibes and García 1984, Sunyer and Viñuela 1994), while the Imperial Eagle is considered a highly specialized predator of rabbits, an abundant and predictable prey across its range (González 1989, Ferrer 1994). Models based on the optimization hypothesis predict that the species with a more irregular and unpredictable food supply should exhibit a more flexible growth (O'Connor 1978). Thus, growth rates should be more variable in Red Kites than in Imperial Eagles.

METHODS

We performed this study at Doñana National Park (SW Spain, 37°N 6°5'W) in 1987 and 1988. We measured 44 Red Kite chicks from 26 nests (12 one-chick, 8 two-chick and six three-chick broods). Most nests were visited daily during hatching, so the ages of the chicks were known within an accuracy of one day. In three cases, we estimated age using a linear regression for growth of the seventh primary, a reliable method for aging raptors (e.g., Juillard 1979, Bortolotti 1984, Viñuela and Bustamante 1992). After hatching, nests were visited every 4–7 days (mostly every 5 days). For every visit and chick we recorded (1) tarsus length from the junction with the central toe to the center of tibio-tarsal joint, measured with Vernier caliper to the nearest 0.1 mm, (2) seventh primary length (sheath plus emerged vane to the nearest mm), and (3) mass, measured with Pesola balances (100, 500 or 1000 g, depending on the size of the chick) to the nearest g.

We collected 40 blood samples from chicks aged 28–54 days by extracting 1–2 cc from the brachial vein (see Viñuela et al. 1991). Blood extractions were made at the same time of day to eliminate possible diurnal fluctuations caused by the circadian rhythm (e.g., García-Rodríguez et al. 1987a, Ferrer et al. 1994). The blood was kept cold in heparin-lithium tubes. Centrifuging and separation of plasma (10 min at 3000 G) was completed within 24 hours of extraction, and the sample was kept at 4° C until it was analyzed. Chemical analyses were made in an automatic analyser (Hitachi 705) at the Valme Hospital (Sevilla, Spain). We determined the blood levels of (1) alkaline phosphatase, an enzyme that is intimately linked to ossification and growth of the skeleton (e.g., Fleisch and Newman 1961, Krabbe and Christiansen 1984, Salo et al. 1986, Kan and Cress 1987, Viñuela et al. 1991) and (2) urea, a good indicator of protein catabolism associated with fasting conditions (Sturkie 1976, García-Rodríguez et al. 1987b, Ferrer, 1993). Levels of alkaline phosphatase show significant variation associated with the age of the chicks (Viñuela et al. 1991). To avoid the possible confounding effects induced by this relationship, we used the residuals from the regression of alkaline phosphatase levels and chick age (relatively high or low alkaline phosphatase levels for a given age). Urea levels were not significantly affected by age ($r = -0.19$, $P = 0.24$, $N = 40$).

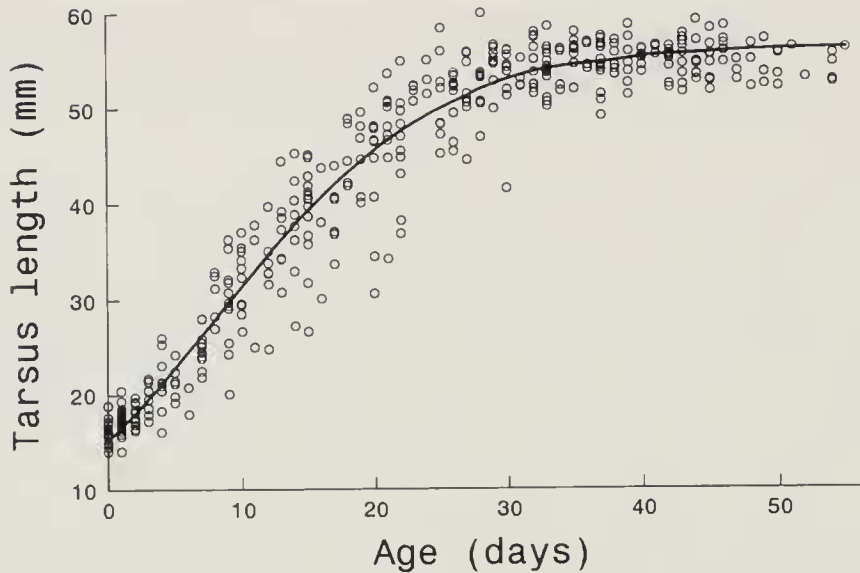


FIG. 1. Tarsus length growth of chicks of Red Kites. Logistic curve shown.

Growth of birds is usually described by means of one of three sigmoid models: the logistic, Von Bertalanffy, or Gompertz equations (O'Connor 1984). The Richards curve may be a more valuable technique, since it is a flexible-shaped model that may explain higher percentages of variance and produce less biased estimates of growth parameters (White and Brisbin 1980, Brisbin et al. 1987, Leberg et al. 1993), but its use in the study of growth of birds has been strongly criticized (Zach 1988). Fixed-shaped growth curves fitting requires the estimation of three growth parameters, while the Richards curve requires four. Consequently, the fixed-shaped models require fewer data for meaningful fitting and are less demanding in terms of data precision (Zach 1988). This has precluded the use of the Richards curve in the study of growth of raptors, where the use of fixed-shaped models is the rule (see e.g., Donazar and Ceballos 1989, Steidl and Griffin 1991, Wiebe and Bortolotti 1994). However, Leberg et al. (1993) presented a form of the Richards curve requiring the estimation of only three parameters, whenever a good estimate of initial size is provided. We have estimated initial size of tarsus from the sample of chicks measured on the day of hatching ($\bar{x} \pm \text{SD} = 16.2 \pm 1.4$, $N = 31$). We fitted tarsus growth data from all fledged chicks (Fig. 1) to logistic, von Bertalanffy, Gompertz, and Richards models by means of a non-linear least-squares regression routine (Veiga and Hiraldo 1990, Steidl and Griffin 1991). The logistic curve provided the best fitting, as indicated by the residual mean square error (Steidl and Griffin 1991), it explained a high percentage of the variance (high R^2 , Veiga and Hiraldo 1990), and it provided the more realistic growth parameters (Table 1, Fig. 1). Gompertz model did not converge satisfactorily, while von Bertalanffy and Richards models had a lower R^2 and more unrealistic estimates of growth parameters (Table 1). This result confirms those previously reached by Veiga and Hiraldo (1990) who found that growth data of more than 75% of chicks of Red Kites fitted to logistic curves. Zach et al. (1984) also found that Richards model failed to explain a significantly larger proportion of the variance than fixed-shaped models.

Because there usually exists a period of mass recession just before fledging (Hiraldo et al. 1990), and many of the blood extractions were made at this stage of mass recession, we fit mass data with a polynomial curve (Konarzewski and Taylor 1989) (Fig. 1). We included in this curve only the data from chicks 28–54 days old, which encompassed part of the lineal growth stage and final mass recession (when the blood samples were taken).

TABLE 1
TARSUS LENGTH GROWTH PARAMETERS AND NON-LINEAR REGRESSION STATISTICS FOR LOGISTIC (L), GOMPertz (G), VON BERTALANFFY (VB), AND RICHARDS (R) CURVES

| | L | G | VB | R ^a |
|--------------------------------|--------|-----------|--------|----------------|
| Asymptotic tarsus | 56 | 55 | 58.1 | 59.9 |
| Growth rate | 0.12 | 0.085 | 0.08 | — |
| Initial tarsus (b) | 15.2 | 27.5 | 0.79 | — |
| Growth period (T) ^b | — | — | — | 35 |
| Residual sum of squares | 4547.3 | 726,437.6 | 5078.3 | 5540 |
| R ² | 0.947 | -7.49 | 0.94 | 0.935 |

^a Shape parameter *m* of Richards curve equal to 0.07, initial size estimated as the average of chicks measured on the day of hatching (16.2).

^b Constant *T* of Richards curve is an estimate of the approximate length of the growth period.

To get values independent of the age of chicks to be used as estimates of short- and long-term growth condition, we computed the following variables: (1) long-term mass growth condition (LTMC), the difference between chick mass on the day of blood extraction and the value predicted at that age from the polynomial regression of mass on age; (2) short-term mass growth condition (STMC), the difference between the mass gained by the chick since the previous visit to the nest (4–7 days before) and the average increase predicted by the polynomial regression of mass on age for that age period; (3) long-term tarsus growth condition (LTTC), the difference between tarsus length on the day of blood extraction and tarsus length predicted by the logistic curve; and (4) short-term tarsus growth condition (STTC), the difference between the growth in tarsus length since the previous visit and the growth predicted by the logistic curve.

We present data on urea levels and the duration of the nestling period for 40 chicks of the population of Spanish Imperial Eagles, recorded in the same study area and for chicks with an age equivalent to that of the chicks of Red Kites sampled (half through end of the nestling period). We followed the same methodology described above but, given the endangered status of Imperial Eagles, nests were visited less frequently so growth data are not available. We define the nestling period as the time that elapsed between hatching and first flight of the chicks (Viñuela and Bustamante 1992). The duration of the nestling period was determined for 35 and 30 chicks of Red Kites and Imperial Eagles, respectively.

Statistical analyses were performed on SPSS. We used quadratic equations when suggested by scatter plots. Quadratic curve fitting was done, using multiple regression analyses including the dependent variable and its square power, and using the conditional sum of squares to assess whether the inclusion of the square power significantly increased the variance explained by the model. When indicated by non-significant *t*-tests, the constant was excluded from the model. To compare the variability of urea levels and the duration of the nestling period among the two species, we used the coefficients of variation and *F*-tests, following the method outlined by Lewontin (1966). Alkaline phosphatase was used as dependent or independent variable depending on the predictions of every analysis (Fig. 2). We predicted that blood levels of alkaline phosphatase could affect growth rate of skeletal structures, so in this case it was considered as independent variable. On the contrary, we predicted that its blood levels could be affected by nutritional condition, as indicated by relative mass growth rate, so in this case it has been considered as dependent variable.

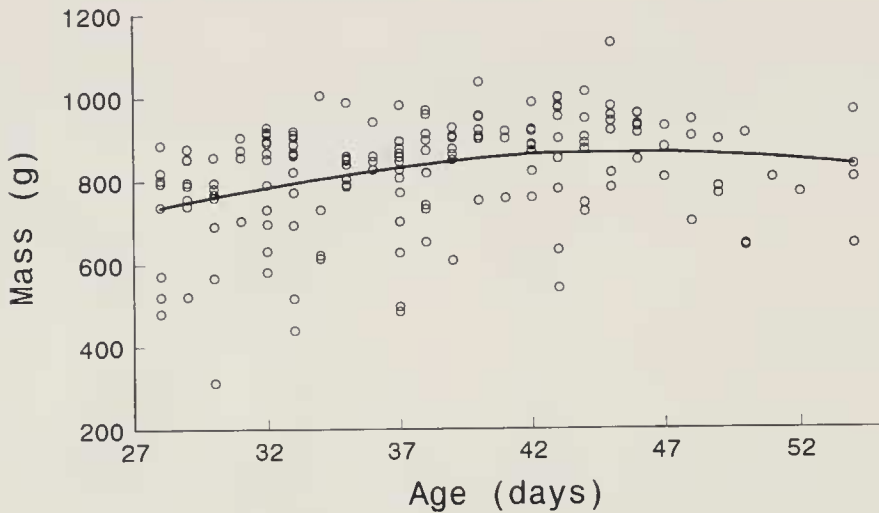


FIG. 2. Mass growth of chicks of Red Kites 28–54 days old (curve shown: $y = 38x - 26.6x^2$, $F = 3957.1$, $P < 0.001$).

RESULTS

We found no significant relationship in Red Kites between long-term tarsus growth (LTTC) or long-term mass growth (LTMC) and relative blood levels of alkaline phosphatase (RAP) ($r = -0.11$, $N = 40$, $P = 0.48$ and $r = -0.08$, $N = 40$, $P = 0.63$, respectively) (Fig. 3). Short-term tarsus growth (STTC) and RAP were correlated significantly (Fig. 3). We found a significant quadratic regression between short-term mass growth condition (STMC) and RAP (Fig. 3). The chicks with the highest and the lowest relative mass increases, respectively showed relatively low levels of alkaline phosphatase. We also found a significant quadratic relationship between STTC and STMC (Fig. 4). Relatively low tarsus length increases were associated with the least and the greatest mass increases. Urea levels were negatively correlated with LTMC ($F = 4.5$, $r = -0.32$, $N = 40$, $P = 0.04$) but not with STMC ($r = -0.12$, $N = 40$, $P = 0.45$).

Urea levels of chicks of Imperial Eagles were higher and more variable than those of Red Kites (Means: 23.37 mg/dl vs 14.85 mg/dl, $t = 2.36$, $P < 0.05$; coefficients of variation: 97.5% vs 55%, $F = 3.17$, $df = 39$, $P < 0.01$). The length of the nestling period was more variable in Red Kites (55.5 ± 5.9 days $N = 35$) than in Imperial Eagles (75.7 ± 4.1 days, $N = 30$) (coefficients of variation: 10.63% vs 5.48%, $F = 3.76$, $df = 34, 29$, $P < 0.01$).

DISCUSSION

We found a relationship between short term tarsus growth and blood levels of alkaline phosphatase. This result was expected, given the im-

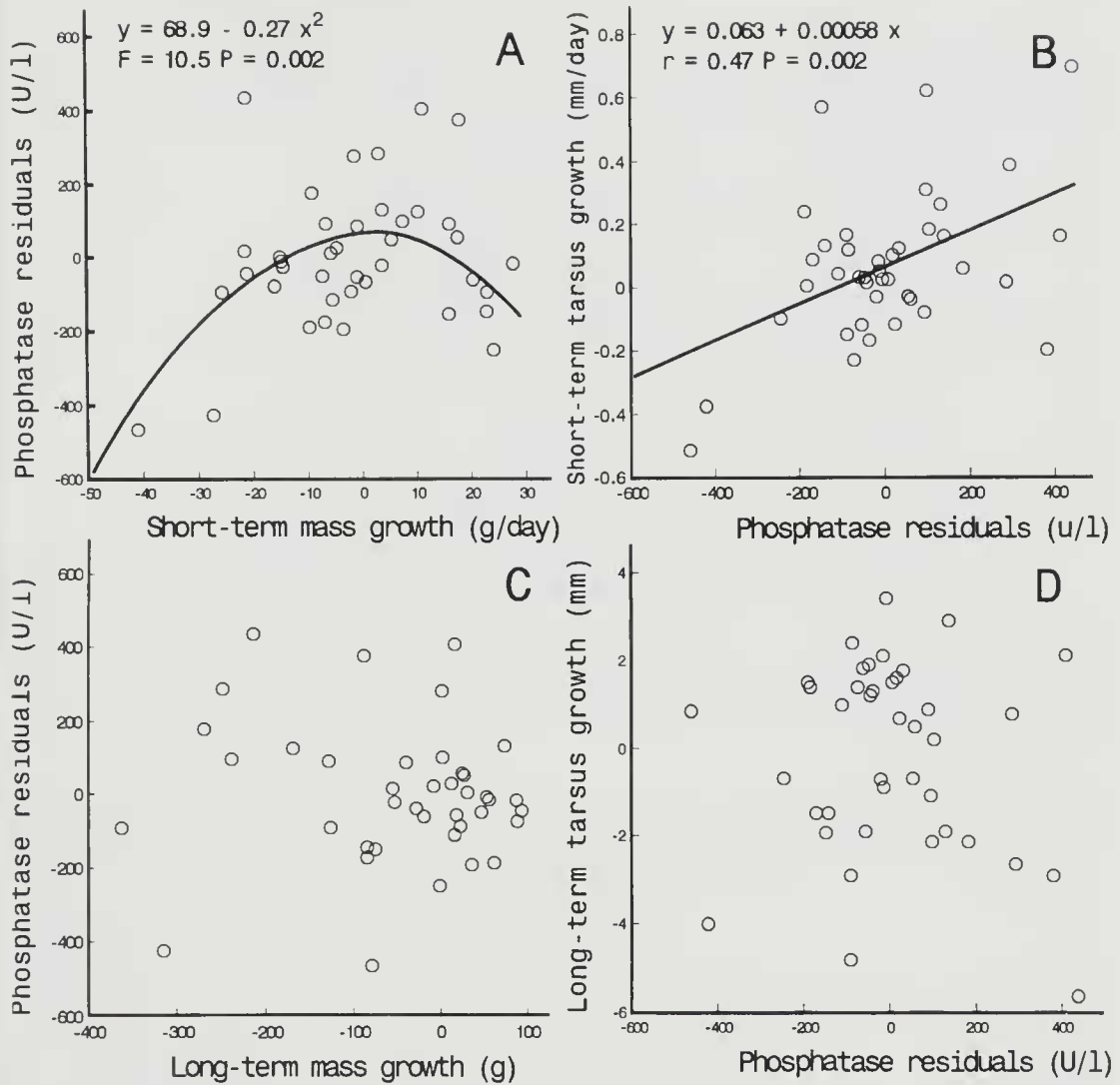


FIG. 3. Plots of short-term mass (A) and short-term tarsus length (B), growth condition variables indicating if the growth during the days before blood extraction was relatively rapid or slow; long-term mass (C) and long-term tarsus length (D) growth condition, indicating if the size on the day of blood extraction was relatively large or small and as dependent (mass plots) or independent variable (tarsus plots) (see Methods), the relative blood levels of alkaline phosphatase (RAP, U/l). No significant relationships were found between RAP and long-term growth variables. See Methods for detailed definitions of the variables.

portant role played by this enzyme in the growth and mineralization of skeletal structures (Krabbe and Christiansen 1984, Kan and Cress 1987, Salo et al. 1986). However, levels of alkaline phosphatase were not correlated with variables indicating long-term growth condition. This suggests that changes in blood levels of this enzyme and the associated growth rate may be rapid responses to food availability. We found some chicks showing relatively high mass growth but slow tarsus growth and relatively low levels of alkaline phosphatase. This situation must corre-

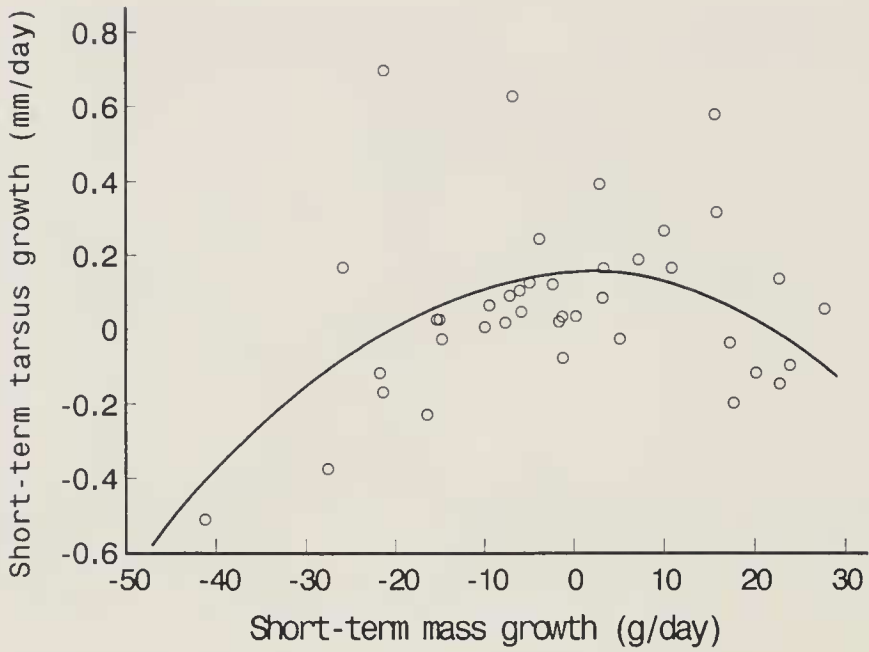


FIG. 4. Relationship between short-term tarsus growth and short-term mass growth (see Fig. 2; curve shown: $y = 0.15 - 0.0003x^2$, $F = 10.1$, $P = 0.003$).

spond to chicks recovering mass after a period of fasting, as we found a negative correlation between individual mass growth rates and short term mass growth ($r = -0.5$, $N = 30$, $P < 0.005$). Thus, the greatest mass increases were detected in chicks that suffered prolonged fasting periods. These results suggest that, after a period of fasting, there could be a delay in the recovery of normal blood levels of alkaline phosphatase, the chicks quickly recovering mass before resuming skeleton growth at the normal rate. Also, this lends support to the existence of compensatory growth after fasting periods, as has been recently demonstrated in an experiment with captive breeding American Kestrels (*Falco sparverius*; Negro et al. 1994).

The decrease in the growth rates of Red Kite chicks under conditions of food scarcity could reduce metabolic expenses and explain why we did not find any relationship between short-term growth rates and urea levels. However, urea levels were negatively correlated with long-term mass condition. Chicks with more delayed growth (lowest long-term mass condition values) are probably those suffering more protracted previous fasting periods, those in which protein catabolism had to be activated to guarantee survival during a long fasting period (high urea levels).

Variation in the duration of the nestling period mainly reflects variation in growth rates (Bortolotti 1986, Viñuela and Bustamante 1992). Growth rates of chicks of Imperial Eagles were less variable, but as indicated by urea levels, their metabolism was also more sensitive to situations of food

scarcity. These results suggest that the ability of the chicks to reduce growth rate to provide energy savings for self-maintenance is better developed in Red Kites than in Imperial Eagles. A well-developed ability to retard growth may be especially important for chicks of species with a very irregular and unpredictable food supply, such as the Red Kite (Delibes and Garcia 1984, Veiga and Hiraldo 1990). In such a situation, growth works as a 'fine-tuning' system to regulate productivity (Veiga and Hiraldo 1990). In contrast, growth delay would be unfavorable for chicks of a species that feeds on a more regular and predictable food supply but experiences strong intrabrood competition eventually leading to fratricide (Meyburg 1974, González 1989). Consequently, our results support the Growth Optimization Hypothesis and agree with the prediction by O'Connor (1978) that species feeding on more unpredictable and irregular food sources should have a more flexible growth pattern.

We do not know if the regulation of alkaline phosphatase blood levels in the Red Kite is active or passive, as defined by Callow and Townsend (1981). The levels of this enzyme may be regulated by growth hormone or by other related hormones (Brown et al. 1983, Kan and Cress 1987), supporting the existence of active regulation. Alkaline phosphatase is essential to the process of skeletal growth, as opposed to mass growth, and is susceptible to hormonal control. It could be, as suggested by our results, a key physiological mechanism of growth regulation.

ACKNOWLEDGMENTS

F. Recio helped with blood analyses and J. Bustamante provided the data on fledging age of Red Kites. José Pablo Veiga, Juan Moreno, Luis M. Carrascal, Steven R. Beissinger, I. Lehr Brisbin Jr., and an anonymous referee provided useful criticism on previous drafts. This is a contribution to the research project of DGICYT PB87-0405. Financial support to J. Viñuela was provided by a PFPI predoctoral fellowship of MEC. Financial support to J. Viñuela during the last stage of writing was provided by a Fulbright Postdoctoral Fellowship and by NSF grant No. IBN-9407349 to Steven R. Beissinger.

LITERATURE CITED

- BORTOLOTTI, G. 1984. Criteria for determining age and sex of nestling Bald Eagles. *J. Field Ornithol.* 55:467-481.
- . 1986. Evolution of growth rates in eagles: sibling competition vs. energy considerations. *Ecology* 67:182-194.
- BRISBIN, I. L. JR., C. T. COLLINS, G. C. WHITE, AND D. A. MCCALLUM. 1987. A new paradigm for the analysis and interpretation of growth data: the shape of things to come. *Auk* 104:552-554.
- BROWN, R. D., C. C. CHAO, AND L. W. FAULKNER. 1983. Hormone levels and antler development in white-tailed and sika fawns. *Comp. Biochem. Physiol.* 75A:385-390.
- CALLOW, P. AND C. R. TOWNSEND. 1981. Resource utilization in growth. Pp. 220-240 *in* *Physiological ecology: an evolutionary approach to resource use* (C. R. Townsend and P. Callow, eds.). Blackwell, Oxford, England.

- CASE, T. J. 1978. On the evolution and adaptive significance of post-natal growth rates in terrestrial vertebrates. *Q. Rev. Biol.* 55:243–282.
- CHEREL, Y., J.-C. STAHL, AND Y. LE MAHO. 1987. Ecology and physiology of fasting in King Penguin chicks. *Auk* 104:254–262.
- DELIBES, M. AND L. GARCÍA. 1984. Hábitos alimenticios del milano real en Doñana durante el periodo de cria. *Ardeola* 31:115–121.
- DONÁZAR, J. A. AND O. CEBALLOS. 1989. Growth rates of nestling Egyptian vultures *Neophron percnopterus* in relation to brood size, hatching order and environmental factors. *Ardea* 77:216–226.
- FERRER, M. 1993. Blood chemistry studies in birds: some applications in ecological problems. Pp. 1031–1044 in *Trends in comparative biochemistry and physiology* (S. G. Pandalai, ed.). Research Trend, Council of scientific research integration, Trivandrum, India.
- . 1994. El Aguila Imperial. Quercus, Madrid, Spain.
- , J. A. AMAT, AND J. VIÑUELA. 1994. Daily variations of blood chemistry values in the Chinstrap Penguin (*Pygoscelis antarctica*) during the Antarctic summer. *Comp. Biochem. Physiol.* 107A:81–84.
- FLEISCH, H. AND W. F. NEWMAN. 1961. Mechanism of calcification: role of collagen, polyphosphates and phosphatase. *Am. J. Physiol.* 200:1296–1300.
- GARCÍA-RODRÍGUEZ, T., M. FERRER, F. RECIO, AND J. CASTROVIEJO. 1987a. Circadian rhythms of determined blood chemistry values in Buzzards and Eagle owls. *Comp. Biochem. Physiol.* 88A:663–669.
- , ———, J. C. CARRILLO, AND J. CASTROVIEJO. 1987b. Metabolic responses of *Buteo buteo* to long-term fasting and refeeding. *Comp. Biochem. Physiol.* 87A:381–386.
- GONZÁLEZ, L. M. 1989. Historia Natural del Aguila Imperial Ibérica (*Aquila adalberti*). Ph. D. diss., Universidad Complutense de Madrid, Madrid, Spain.
- HIRALDO, F., J. P. VEIGA, AND M. MAÑEZ. 1990. Growth of nestling Black Kites *Milvus migrans*: effects of hatching order, weather and time of season. *J. Zool. (Lond.)* 222:197–214.
- JUILLARD, M. 1979. La croissance des jeunes Chouettes cheveches, *Athene noctua*, pendant leur séjour au nid. *Nos Oiseaux* 35:113–124.
- KAN, K. W. AND R. L. CRESS. 1987. Temporal relationship between fetal bovine skeletal growth and circulating hormone levels. *Calcif. Tissue Int.* 40:137–148.
- KONARZEWSKI, M. AND J. R. E. TAYLOR. 1989. The influence of weather conditions on growth of Little Auk *Alle alle* chicks. *Ornis Scand.* 20:112–116.
- KRABBE, S. AND C. CHRISTIANSEN. 1984. Longitudinal study of calcium metabolism in male puberty. II. Relationship between mineralization and serum testosterone. *Acta Paediatr. Scand.* 73:750–755.
- LEBERG, P., M. H. SMITH, AND I. L. BRISBIN JR. 1993. Influence of sex, habitat, and genotype on the growth patterns of White-tailed deer. Pp. 343–350 in *The biology of deer* (R. D. Brown, ed.). Springer-Verlag, Berlin.
- LEWONTIN, R. C. 1966. On the measurement of relative variability. *Syst. Zool.* 15:141–142.
- MEYBURG, B.-U. 1974. Sibling aggression and mortality among nestling eagles. *Ibis* 116:224–228.
- NEGRO, J. J., A. CHASTIN, AND D. M. BIRD. 1994. Effects of short-term food deprivation on growth of hand-reared American Kestrels. *Condor* 96:749–760.
- O'CONNOR, R. J. 1978. Growth strategies in nestling passerines. *Living Bird* 16:209–238.
- . 1984. *The growth and development of birds*. Wiley and sons, Chichester, United Kingdom.
- RICKLEFS, R. E. 1968. Patterns of growth in birds. *Ibis* 110:419–451.

- . 1983. Avian postnatal development. Pp. 1–83 in *Avian biology*, Vol. VII (D. S. Farner, ed.). Academic Press, New York.
- . 1984. The optimization of growth rate in altricial birds. *Ecology* 65:1602–1616.
- SALO, L. A., T. YLINIEMI, M. LARMAS, AND M. NIEMINEN. 1986. Phosphatase and peptidase activities in reindeer antler throughout the growth cycle. *J. Interdiscipl. Cycle Res.* 17: 1–6.
- STEIDL, R. J. AND C. R. GRIFFIN. 1991. Growth and brood reduction of mid-Atlantic coast ospreys. *Auk* 108:363–370.
- STURKIE, P. D. (Ed.). 1976. *Avian physiology*. Springer, New York, New York.
- SUNYER, C. AND J. VIÑUELA. 1994. Variación temporal en los hábitos alimentarios del Milano real durante la invernada en la meseta norte. *Ardeola* 41:161–167.
- VEIGA, J. P. AND F. HIRALDO. 1990. Food habits and the survival and growth of nestlings in two sympatric kites (*Milvus milvus* and *Milvus migrans*). *Holarctic Ecol.* 13:62–71.
- VIÑUELA, J., M. FERRER, AND F. RECIO. 1991. Age-related variations in plasma levels of alkaline phosphatase, calcium and inorganic phosphorus in chicks of two species of raptors. *Comp. Biochem. Physiol.* 99A:49–54.
- AND J. BUSTAMANTE. 1992. Effect of growth and hatching asynchrony on the fledging age of black and red kites. *Auk* 109:748–757.
- WHITE, G. C. AND I. L. BRISBIN JR. 1980. Estimation and comparison of parameters in stochastic growth models for Barn Owls. *Growth* 44:97–111.
- WIEBE, K. L. AND G. R. BORTOLOTTI. 1994. Energetic efficiency of reproduction: the benefits of asynchronous hatching for American Kestrels. *J. Anim. Ecol.* 63:551–560.
- ZACH, R. 1988. Growth-curve analysis: a critical reevaluation. *Auk* 105:208–210.
- , Y. LINER, G. L. RIGBY, AND K. R. MAYOH. 1984. Growth curve analysis of birds: the Richards model and procedural problems. *Can. J. Zool.* 62:2429–2435.